Glutathione *S*-Transferase Mu and Theta Polymorphisms and Breast Cancer Susceptibility

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Background: **The enzymes encoded by the glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) genes are involved in the metabolism (mainly inactivation, but activation is possible) of a wide range of carcinogens that are ubiquitous in the environment; the enzyme encoded by the GSTT1 gene may also be active in endogenous mutagenic processes. Homozygous deletions of the GSTM1 and GSTT1 genes are commonly found in the population and result in a lack of enzyme activity. This study was undertaken to evaluate the association between GSTM1 and GSTT1 gene polymorphisms and breast cancer risk.** ***Methods:*** **Our study included 466 women with incident cases of breast cancer occurring from May 1989 through May 1994 and 466 matched control subjects. These individuals were part of a prospective cohort of U.S. women (i.e., the Nurses' Health Study). Odds ratios (ORs) and 95% confidence intervals (CIs) from conditional logistic regression models were used to estimate the association between genetic polymorphisms and breast cancer risk.** ***Results:*** **The GSTM1 and GSTT1 null genotypes were not associated with an increased risk of breast cancer (OR = 1.05 [95% CI = 0.80–1.37] for GSTM1 null; OR = 0.86 [95% CI = 0.61–1.21] for GSTT1 null). On the contrary, a suggestion of a decreased risk of breast cancer associated with the GSTT1 null genotype was observed among premenopausal women. When considered together, no combination of the GSTM1 and GSTT1 genotypes was associated with an increased risk of breast cancer. The relationship between GSTM1 and GSTT1 gene deletions and breast cancer risk was not substantially modified by cigarette smoking.** ***Conclusions:*** **Our data provide evidence against a substantially increased risk of breast cancer associated with GSTM1 and/or GSTT1 homozygous gene deletions. [J Natl Cancer Inst 1999;91:1960–4]**

Recognized risk factors for breast cancer cannot fully explain the observed variation in breast cancer incidence over time and across geographic locations *(1,2)*. Environmental carcinogens, such as polycyclic aromatic hydrocarbons, could be responsible for some of the unexplained variation *(3,4)*. Many chemical carcinogens are activated or inactivated through metabolic reactions. Genetically determined differences in the activity of metabolizing enzymes involved in these reactions might contribute to host susceptibility to cancer; thus, taking these genetic factors into account may improve our ability to determine if environmental chemicals contribute to breast cancer *(5)*.

The glutathione *S*-transferase mu (GST-M1) and theta (GST-T1) are separate isoforms of glutathione transferase enzymes that participate in the metabolism of a wide range of chemicals, including possible carcinogens *(6)*. The known substrates for the GST-M1 enzyme in-

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clude reactive epoxide intermediates generated from the activation of polycyclic aromatic hydrocarbons by cytochrome P450 enzymes *(6)*. Exposure to polycyclic aromatic hydrocarbons from cigarette smoke and other sources is ubiquitous and has been shown to induce mammary tumors in animal models *(7)*. GST-T1 enzyme substrates include chemicals with wide industrial use that can also cause mammary tumors in animals *(7);* such chemicals include methyl chloride (a methylating agent), methyl bromide (a soil fumigant), ethylene oxide (a widely used agent for sterilization and an intermediate product in the production of polyester fibers and non-ionic surfactants), and dichloromethane (a solvent).

Homozygous deletions in the genes coding for the enzymes GST-M1 (GSTM1 gene) and GST-T1 (GSTT1 gene) are commonly found in the population, result in the absence of enzyme activity, and have been associated with cancer susceptibility *(8)*. Deficiencies in GST-M1 or GST-T1 enzyme activity have been shown to be associated with increased sensitivity to induction of sister chromatid exchanges in lymphocytes upon exposure to specific mutagenic substrates *(9–12)* or among smokers *(13)*. In addition, baseline sister chromatid exchange frequency in lymphocytes has been found to be higher in individuals with deleted GSTT1 gene *(10)*. Thus, a lack of GST-M1 or GST-T1 enzyme activity could increase the risk for DNA damage from genotoxic substrates. However, GST-T1 enzyme activity does not always result in detoxification; it can also yield mutagenic metabolites, such as conjugated metabolites of dihalomethanes (e.g., dichloromethane) *(14,15)* and dihaloethanes (e.g., ethylene dibromide) *(16)*.

Because of the potential carcinogenic effects of some of the GST-M1 and GST-T1 enzyme substrates and the possibility that the GSTT1 gene deletion is associated with enhanced endogenous mutagenic processes, the GSTM1 and GSTT1 gene polymorphisms could be important in human carcinogenesis. The relationship between these polymorphisms and breast cancer risk has been evaluated in several small case–control studies, with contradictory results *(17–19)*. In a nested case–control study among 110 U.S. Caucasian case patients and 113 control subjects, Helzlsouer et al. *(20)* reported a statistically significant 2.1-fold and a statistically nonsignificant 1.5-fold increase in risk of breast cancer for the homozygous deletion in the GSTM1 and GSTT1 genes, respectively. Finally, a recent study of 361 case patients and 437 control subjects in France *(21)* reported a twofold increase in breast cancer risk associated with the GSTM1 null genotype among women older than 50 years of age.

We originally evaluated the relationship between breast cancer risk and the GSTM1 gene polymorphism (but not the GSTT1 gene polymorphism) among 240 pairs of case patients and control subjects nested within a prospective cohort of U.S. women (the Nurses' Health Study) *(22);* in this analysis, the case patients had been diagnosed from the time of blood collection (i.e., from May 1989 through December 1990) through May 31, 1992. We did not observe a material increase in breast cancer risk associated with the GSTM1 null genotype (odds ratio [OR] = 1.08; 95% confidence interval [CI] = 0.74–1.57). In this study, we evaluate the association between GSTM1 and GSTT1 gene polymorphisms and breast cancer risk among 466 case–control pairs that include the 240 case patients diagnosed before June 1, 1992, and 226 new case patients diagnosed from June 1, 1992, through May 31, 1994.

Materials and Methods

Study Population

In 1976, a total of 121 700 U.S. female registered nurses between ages 30 and 55 years, residing in 11 U.S. states, completed a mailed questionnaire, forming the basis for the Nurses' Health Study cohort *(23)*. The baseline questionnaire collected information on potential risk factors for breast cancer, including smoking habits early in life. Updated information on potential risk factors and identification of new cases of disease have been ascertained every 2 years through mailed questionnaires. Self-reported cases of breast cancer are confirmed by medical record review.

From May 1989 through December 1990, we collected blood samples from 32 826 participants in the Nurses' Health Study cohort, as previously described *(24)*. The women who sent blood and did not have a diagnosis of cancer (except for nonmelanoma skin cancer) at that time served as the base population for a nested case–control study of breast cancer. Eligible case patients were women from this subcohort who had a confirmed diagnosis of breast cancer after blood collection and before June 1, 1994. During this period, 466 eligible case patients with breast cancer were identified. For each case patient, a control subject was chosen among women in the subcohort who had not developed cancer at the time of diagnosis of the case patient. Control subjects were matched to the case patients on year of birth, postmenopausal hormone use, time of day of blood collection, month of blood return, and fasting status at blood collection. Matching factors were selected to increase the study efficiency to control for potential confounding factors in the analysis of blood biomarkers, particularly levels of hormones (not included in this report). Matching was taken into account in the analysis by using conditional logistic regression models for matched sets; therefore, matching should not have affected the validity of our findings. The study protocol was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital, and informed consent was obtained from all of the participants.

Laboratory Methods

Upon arrival in our laboratory, blood samples were centrifuged at 750*g* for 20 minutes at 4 °C and separated into plasma, buffy coat, and red blood cells. Since the time of collection, blood components have been archived in continuously monitored, liquid nitrogen freezers. Genotypes for the GSTM1 and GSTT1 deletions were determined by polymerase chain reaction (PCR) on genomic DNA (Chelex extraction kit [Sigma Chemical Co., St. Louis, MO] or buffy coat extraction kit [Qiagen, Chatsworth, CA]), with the use of methods published previously *(10,25)*. Briefly, a PCR solution with primers hybridizing to the 5′ region of exon 4 (5′-CTGCCCTACTTGATTGATGGG-3′) and the 3′ region of exon 5 (5′-CTGGATTGTAGCAGATCATGC-3′) of GSTM1 was used to amplify a 273-base-pair (bp) fragment *(25)*. Control primers that amplify β-actin (493 bp) were also included in each reaction to confirm the presence of amplifiable DNA in the samples. Similarly, a PCR solution with primers for the 3′-coding region of the human GSTT1 (5′-TTCCTTACTGGTCCTCACATCTC and 5′-TCACCGGATCATGGCCAGCA) was used to amplify a 480-bp fragment *(10)*. In both assays, the absence of the PCR product was indicative of the null genotype (homozygous deletion). These assays do not distinguish between heterozygous and homozygous wild-type genotypes. Laboratory personnel were blinded to case–control status, and 10% repeat samples were included in the PCR analysis to monitor quality control. All repeat samples for both the GSTM1 and GSTT1 genotypes were concordant.

Statistical Analysis

Chi-squared tests for contingency tables were used to assess differences in genotype prevalence across different groups of women. ORs and 95% CIs were used to estimate the association between the GSTT1 and GSTM1 genotypes and breast cancer risk. ORs adjusted for potential confounders were estimated with conditional logistic regression models for matched sets. Age at menarche, family history of breast cancer among mother or sisters, parity, age at first live birth, body mass index (weight in kilograms divided by [height in meters]^2), and benign breast disease were considered as potential confounders. Gene–gene and gene–environment interactions were assessed in logistic regression models by including indicator variables for each category defined by the cross-classification of the interacting variables, except for the reference category. A likelihood ratio test was used to test for multiplicative interactions. To assess modification of the effect by matching variables (menopausal status and age), we included a term for the genotype and an interaction term between the matching variable and the geno-

type in a conditional logistic model. All *P* values reported are two-sided.

RESULTS

This study included 466 case–control pairs matched on age at blood return, postmenopausal hormone use, time of day of blood collection, and month of blood return; 97% of the case patients and 95% of the control subjects were Caucasians. The mean age of both case patients and control subjects was 58 (±7) years. Of 466 case patients, 78 (17%) women were premenopausal, 358 (77%) were postmenopausal, and 30 (6%) had uncertain menopausal status. We obtained complete histologic information on 465 case patients. Among these cases, 392 (84%) were invasive tumors and 73 (16%) were *in situ* tumors. Among the 392 invasive tumors, 333 (85%) were ductal, 42 (11%) were lobular, 15 (4%) had both ducts and lobules involved, and two had inconclusive histology. Differences in established risk factors for breast cancer among case patients and control subjects in this study were mostly in the expected direction *(24)*. In brief, case patients had slightly higher age at first live birth and were more likely to have a history of benign breast disease and a family history of breast cancer.

The prevalence of the GSTM1 homozygous deletion was similar for case patients and control subjects (50% and 49%, respectively), whereas a slightly lower frequency of case patients than control subjects had the GSTT1 homozygous deletion (15% and 17%, respectively). After adjusting for matching variables, we observed no evidence for an association between the GSTM1 deletion and breast cancer risk, and we found a suggestion of an inverse association for the GSTT1 deletion (Table 1). Further adjustment for other potential confounders did not substantially change the estimated ORs; therefore, we present only estimates adjusted for matching variables.

We observed a stronger inverse association between breast cancer risk and GSTT1 homozygous deletion among premenopausal women than among postmenopausal women (Table 1) (test for interaction: $\chi^2_{(1)} = 5.32$; $P = .02$). No evidence of a statistically significant heterogeneity of the OR for the GSTM1 or GSTT1 homozygous deletions was observed when we stratified women as being younger than 60 years or 60 years old or older (data not shown).

We examined different histologic types of postmenopausal (n = 358) breast cancer separately. The frequency of GSTT1 deletion was similar for patients with *in situ* carcinomas (five [14%] of 37) and invasive tumors (52 [16%] of 318) ($P = .65$). Among patients with invasive tumors, the GSTT1 deletion was more common among women with lobular tumors (10 [26%] of 39) than among women with ductal tumors (40 [15%] of 265); however, this difference was not statistically significant ($P = .10$). Analysis of premenopausal tumors by histologic type was not possible because of sparse data.

We explored a possible interaction between the GSTT1 and GSTM1 genotypes among all women and among postmenopausal women only (Table 2). Neither women with a deletion in one gene only nor women with a deletion in both genes were at increased risk of breast cancer. We were unable to explore this interaction among premenopausal women because of the small number of women in this group.

We examined the interaction between the GSTM1 and GSTT1 null genotypes and cigarette smoking defined immediately prior to diagnosis, 10 years prior to diagnosis, as pack-years, and according to duration of smoking prior to first pregnancy. Similar to the previously published data from the first 240 incident case patients *(22)*, we found no evidence of a multiplicative interaction between the GSTM1 null genotype and any of the smoking variables considered, for the group as a whole or for postmenopausal women specifically. Although there was some indication of an increase in risk of breast cancer among postmenopausal women with the GSTT1 present genotype and a lifetime exposure to 20 or more pack-years of cigarettes, our data provided no evidence for a modification of the effect of pack-years by the GSTT1 null genotype (Table 3). We also did not observe a multiplicative interaction between the GSTT1 null genotype and any of the other smoking variables considered (data not shown). We were unable to adequately explore gene–smoking interactions among premenopausal women because of the small sample size.

DISCUSSION

We did not find evidence for an association between the GSTM1 or the GSTT1 null genotypes, alone or in combination, and an increased risk for developing breast cancer. In fact, the frequency

Table 1. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast cancer risk and glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) null deletion genotypes, stratified by menopausal status*

	Total			Premenopausal			Postmenopausal		
Genotype	No. of case patients (%) (n = 466)	No. of control subjects (%) (n = 466)	OR (95% CI)†	No. of case patients (%) (n = 78)	No. of control subjects (%) (n = 86)	OR (95% CI)†	No. of case patients (%) (n = 358)	No. of control subjects (%) (n = 348)	OR (95% CI)†
GSTM1									
Present	233 (50.1)	237 (51.1)	1.0 (referent)	37 (47.4)	41 (47.7)	1.0 (referent)	179 (50.1)	177 (51.2)	1.0 (referent)
Deleted	232 (49.9)	227 (48.9)	1.05 (0.80–1.37)	41 (52.6)	45 (52.3)	0.92 (0.42–2.02)	178 (49.9)	169 (48.8)	1.06 (0.78–1.45)
GSTT1									
Present	396 (85.0)	386 (82.8)	1.0 (referent)	69 (88.5)	68 (79.1)	1.0 (referent)	301 (84.1)	288 (82.8)	1.0 (referent)
Deleted	70 (15.0)	80 (17.2)	0.86 (0.61–1.21)	9 (11.5)	18 (20.9)	0.23 (0.07–0.81)	57 (15.9)	60 (17.2)	0.90 (0.59–1.35)

*One case patient (postmenopausal) and two control subjects (postmenopausal) are excluded from this table because of missing data for GSTM1 genotype; 30 case patients and 32 control subjects with uncertain menopausal status were excluded from the analysis by menopausal status.

†Conditional logistic regression analysis on case–control pairs matched on current age, postmenopausal hormone use, time of day of blood collection, and month of blood return. Test for multiplicative interaction between genotypes and menopausal status: $\chi^2_{(1)} = 0.79$ and $P = .37$ for GSTM1, and $\chi^2_{(1)} = 5.32$ and $P = .02$ for GSTT1.

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast cancer risk and the combination of glutathione *S*-transferase mu 1 (GSTM1) and theta 1 (GSTT1) null deletion genotypes*

Genotype		Total			Postmenopausal		
GSTM1	GSTT1	No. of case patients (%) (n = 465)	No. of control subjects (%) (n = 464)	OR (95% CI)†	No. of case patients (%) (n = 357)	No. of control subjects (%) (n = 346)	OR (95% CI)†
Present	Present	198 (42.6)	192 (41.4)	1.0 (referent)	148 (41.5)	142 (41.0)	1.0 (referent)
	Deleted	35 (7.5)	45 (9.7)	0.76 (0.47–1.24)	31 (8.7)	35 (10.1)	0.81 (0.46–1.43)
Deleted	Present	197 (42.4)	192 (41.4)	1.00 (0.74–1.34)	152 (42.6)	144 (41.6)	1.02 (0.71–1.43)
	Deleted	35 (7.5)	35 (7.5)	0.98 (0.59–1.64)	26 (7.3)	25 (7.2)	1.04 (0.56–1.92)

*One case patient and two control subjects are excluded from this table because of missing data for GSTM1 genotype.

†Conditional logistic regression analysis with case–control pairs matched on current age, postmenopausal hormone use, time of day of blood collection, and month of blood return. Test for multiplicative interaction between GSTM1 and GSTT1 genotypes: $\chi^2_{(1)} = 0.10$ and $P = .75$ among all women, and $\chi^2_{(1)} = 0.03$ and $P = .86$ among postmenopausal women.

of the GSTT1 null genotype was slightly lower for case patients than for control subjects, especially among premenopausal women.

In a nested case–control study with 110 case patients and 113 control subjects, Helzlsouer et al. *(20)* reported a statistically significant 2.5-fold increase in postmenopausal breast cancer risk associated with the GSTM1 homozygous deletion and a statistically nonsignificant increase in risk associated with the GSTT1 null genotype among both premenopausal and postmenopausal women. Results from this study suggested an increasing risk of breast cancer associated with increasing number of susceptibility genotypes for GSTM1, GSTT1, and GSTP1 (i.e., glutathione *S*-transferase pi). Although we did not evaluate the GSTP1 genotype, our data do not support an increasing risk of breast cancer associated with increasing number of null genotypes for the GSTM1 and GSTT1. Similar to our study, a case–control study of 164 U.S. Caucasian and 59 African-American case patients *(19)* found no evidence for an increased risk of premenopausal or postmenopausal breast cancer associated with the CYP1A1, GSTM1, and GSTT1 genotypes, either alone or in combination. Two studies *(17,18)* on postmenopausal Caucasians that evaluated the GSTM1 null genotype also failed to report an association with breast cancer risk, with the possible exception of a suggestion of an increase in risk among young postmenopausal women in the study of Ambrosone et al. *(17).* In contrast, a recent study in France *(21)* reported a twofold increase in breast cancer risk associated with the GSTM1 null genotype among women older than 50 years of age. Participants in our study were selected among women participating in the Nurses' Health Study prospective cohort who sent a blood specimen in 1989–1990. The distribution of reproductive risk factors, such as age at menarche, parity, and age at first live birth, was very similar for women who provided a blood specimen and for women who did not; however, women who provided a blood sample were less likely to be current smokers and more likely to have a history of benign breast disease or a family history of breast cancer. These differences should not compromise the internal validity of the study because of the prospective nature of the study. Moreover, the prevalence of GSTM1 and GSTT1 in our control population is similar to previously published prevalences in other U.S. Caucasian populations *(8,17,19,20),* suggesting that selection factors are unlikely to be related to these genotypes.

The GST-T1 enzyme activity can result in both activation and detoxification reactions; therefore, in principle, the GSTT1 gene deletion could be associated with either an increased or a decreased cancer risk *(6).* We observed a decreased risk of premenopausal breast cancer associated with the GSTT1 null genotype. However, several factors preclude a conclusion about such an association. First, an inverse association with the GSTT1 null genotype is not supported by studies of cytogenetic damage *(9–13)* or by past epidemiologic studies *(19,20).* Second, there is a substantial difference between the crude OR for the GSTT1 null genotype (OR = 0.49; 95% CI = 0.21–1.16) and the matched OR presented in Table 1

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast cancer risk, gluthathione *S*-transferase theta 1 (GSTT1) null deletion genotype, and pack-years of cigarette smoking immediately prior to diagnosis*

		Total			Postmenopausal		
GSTT1	Pack-years	No. of case patients (%) (n = 464)	No. of control subjects (%) (n = 464)	OR (95% CI)†	No. of case patients (%) (n = 356)	No. of control subjects (%) (n = 347)	OR (95% CI)†
Present	0	175 (37.7)	181 (39.0)	1.0 (referent)	121 (34.0)	141 (40.6)	1.0 (referent)
	0 to <20	95 (20.5)	93 (20.0)	1.07 (0.75–1.51)	69 (19.4)	61 (17.6)	1.38 (0.89–2.12)
	⩾20	125 (26.9)	110 (23.7)	1.18 (0.84–1.66)	110 (30.9)	85 (24.5)	1.48 (1.00–2.18)
Deleted	0	23 (6.0)	39 (8.4)	0.61 (0.35–1.06)	20 (5.6)	30 (8.6)	0.71 (0.38–1.34)
	0 to <20	15 (3.2)	19 (4.1)	0.81 (0.40–1.64)	12 (3.4)	12 (3.5)	1.07 (0.42–2.73)
	⩾20	31 (6.7)	22 (4.7)	1.47 (0.83–2.63)	24 (6.7)	18 (5.2)	1.74 (0.88–3.47)

*Two case patients and two control subjects are excluded from this table because of missing smoking data.

†Conditional logistic regression analysis with case–control pairs matched on current age, postmenopausal hormone use, time of day of blood collection, and month of blood return. Test for multiplicative interaction between GSTT1 genotype and pack-years: $P = .24$ among all women and $P = .57$ among postmenopausal women.

(OR = 0.23; 95% CI = 0.07–0.81). This difference could not be explained by the adjustment for matching variables, and it probably reflects instability of our estimates, especially for the matched OR that was based only on four GSTT1 null case patients. Finally, the difference in the GSTT1 OR for premenopausal and postmenopausal women is driven by both a *decrease* in the prevalence of the GSTT1 null prevalence from premenopausal to postmenopausal control subjects (18 [20.9%] of 86 to 60 [17.2%] of 348 [P = .42]) and an *increase* in the prevalence of the GSTT1 null genotype from premenopausal to postmenopausal case patients (nine [11.5%] of 78 to 57 [15.9%] of 358 [P = .33]). These prevalence changes in opposite directions are difficult to explain and are likely due to chance. In summary, our data provide evidence against a substantial increase in risk of breast cancer associated with the GSTM1 and GSTT1 deletions alone or in combination.

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